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Massachusetts General Hospital

LABORATORY MANUAL

FIRST EDITION
1922

MASSACHUSETTS GENERAL HOSPITAL

LABORATORY MANUAL

Prepared by the

WEST MEDICAL SERVICE

Dr. JOSEPH STOKES

Dr. J. EDWIN WOOD

Dr. ISAAC STARR

Dr. LEROY R. WHEELER, Editor

FIRST EDITION

1922

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PREFACE

THIS first edition of a Laboratory Manual intended primarily for use of House Officers of the Massachusetts General Hospital is not absolutely accurate nor, of course, beyond criticism. It is hoped that the colloquialisms and certain provincial abbreviations which have crept into the work will be overlooked by those outside the Hospital. No scientific presentation of the various tests is attempted, but simple statements of the methods which have been found by experience to be sufficiently accurate for ordinary clinical purposes in this Hospital are given.

The Editors wish to express their gratitude to Drs. Roger I. Lee, Wm. H. Smith, and Arlie V. Bock for their helpful suggestions and criticisms.

BOSTON, 1922.

ROUTINE WORK ON ADMISSIONS

(1)

A. All Cases

Day of Admission.

History; physical examination; white count; hemoglobin (Tallqvist); differential; description of red cells and approximate number of platelets. (Report number of platelets by the terms normal, increased, or reduced.)

Subsequently.

Complete urine, stool, blood pressure, and Wassermann, and sputum, if present.

B. Special Cases

Nephritics.

Renal function and N.P.N. on day of admission; daily urines until discontinued by the Senior, and then only when ordered.

Anemias.

Complete blood: white count, red count, hemoglobin, platelets, reticulated reds and smear, bleeding and clotting time, blood group and serum dilution.

Decompensated Cardiacs.

Carry out the directions of the House Officer in charge.

Typhoids.

Widal and blood culture on admission. Guaiacs on all stools.

Pneumonia.

Blood culture, "mouse" the sputum and examine sputum under Smith's stain as soon as case arrives on ward.

Hemorrhage.

Two-hour pulse and blood pressure readings, bleeding and clotting time, blood group. Arrange for donor if indicated.

Diabetes.

Blood sugar on the morning following admission (taken before breakfast). Daily urines, recording the amount, specific gravity, quantitative sugar, and the presence of acetone and diacetic acid.

Hypertension Cases.

Renal function, N.P.N. on admission; blood pressure every 3 days.

Septicemia.

Blood culture.

Acidosis.

Marriott test if possible. If not, blood CO_2. Further instructions from House Officer in charge. Usually these cases are given intravenous glucose.

Gastro-intestinal Hemorrhage.

Daily guaiacs.

Malignant Endocarditis.

Blood culture.

(2) PRECAUTIONS CONCERNING GLASS SYRINGES AND NEEDLES

In testing a syringe do not allow the piston to fly back on the butt end by pulling it out while the outlet is closed with the finger. Do not forcibly discharge the contents of the syringe so that the needle will fly off. Do not let the plunger slide while holding the syringe in the hand. When blood or other viscous material is collected, wash the syringe at once in cold water. Needles must be cleansed and dried first by running water through them, then alcohol, and finally, ether. Wires are always to be replaced in the needles. Before boiling a syringe, cover completely with cold water. After boiling, allow water to cool before emptying sterilizer.

(3) URINE ANALYSIS

The tests described below are those commonly used and are sufficiently accurate for ordinary clinical purposes. In some instances they are at variance with the originator's technique.

Color.

Report as normal clear, normal cloudy, pale clear, pale cloudy, and any unusual colors.

Specific Gravity.

Read from the bottom of the meniscus.

Albumen.

Heller's test:

A wineglass is about one quarter filled with concentrated nitric acid. A filter paper folded into a funnel shape is placed in the wineglass and the urine filtered through. Allow to stand two or three minutes. The albumen ring is gauged by the symbols:

SPT — visible only with dark background.
VST — just visible without dark background.
ST — just visible from above.
T — easily visible from above.
LT — opaque from above.

URINE ANALYSIS—*continued*

Beware of rings that are not exactly at the junction of the urine and nitric acid. These rings are due ordinarily to either urates or phosphates. If in doubt, do a heat and acetic acid test.

Sugar.

To 5 cc. of Benedict's solution add 8 drops of urine and place in the water bath and boil for 5 minutes. If allowed to boil more than 5 minutes, false positives are apt to result.

Acetone.

Legal's test:

To 5 cc. of urine in a test-tube add a few crystals of sodium nitroprusside. Acidify with glacial acetic acid. Shake. Overlay with strong ammonia water. A violet ring at the junction of the two fluids denotes the presence of acetone.

Diacetic Acid.

Gerhardt's test:

To half a test-tube of urine add a slight excess of 10% ferric chloride solution. Filter if a ppt. occurs. In the presence of diacetic acid the urine assumes a Burgundy red color. As many drugs give somewhat similar colors (especially the salicylates) it is sometimes difficult to read the results. It is always safer to divide the urine in halves and boil one half. If the tube assumes a lighter color (due to the breaking down of the diacetic acid) it denotes the presence of diacetic acid. Use the other half as a control.

Bile.

1. Shake one half a test-tube full of urine vigorously and note the color of the foam. This is the routine test and is often sufficient.

2. Pour about 50 cc. of urine through a filter paper. Spread the paper out on a porcelain plate. Drop a drop of concentrated nitric acid on the paper and

URINE ANALYSIS—*continued*

colored concentric rings ranging from pink to green will appear in the presence of bile.

3. Mix one volume of Hammarsten's reagent with four volumes of 95% alcohol. To 5 cc. of this mixture add 1 cc. of urine. A brilliant green color will appear, usually at once, but if the bile is present only in minute quantities, the coloring will be delayed from 10 to 30 minutes.

Melanin.

Place a test-tube of the urine in the sunlight for 12 hours. If there is a perceptible darkening of the urine, it denotes the presence of melanin.

The Schlayer Test.

Apparatus:

100 cc. Ehrlenmeyer flask.
5 cc. pipette.
10 cc. pipette.
Distilled water.
Standard $AgNO_3$ solution. (1 cc. equals .01 NaCl.)
Standard ammonium theocyanate solution. (1 cc. equals 1 cc. of the standard $AgNO_3$ solution.)
Indicator (ferric alum and nitric acid).

Pipette 5 cc. of urine into the flask. Add about 20 cc. of distilled water, 10 cc. of the standard silver nitrate solution, and about 5 cc. of the indicator in the order mentioned. Titrate against the standard ammonium theocyanate solution. The end point is the first permanent red color which appears throughout the contents.

Calculation: Subtract the number of cc. of the ammonium theocyanate solution used from 10 and multiply the result by .01. This gives the amount of sodium chloride in the 5 cc. of urine used. From this calculate the total 24-hr. amount.

URINE ANALYSIS—*continued*

Clean and Sterile Specimens.

A clean specimen is put through the regular routine. On sterile specimens do an albumen, sediment, and culture on bouillon and plain agar.

Quantitative Sugar.

Pipette 25 cc. of Benedict's quantitative solution in an Ehrlenmeyer flask and add 2 dr. of sodium bicarbonate. Fill a burette with urine which has been diluted either 1:5 or 1:10. Arrange the burette so that it delivers the urine directly into the flask, which has been placed on a tripod over a flame. Bring the Benedict's solution to a boil and add at first quickly and then slowly the urine until all the Benedict's solution is reduced. The solution must boil gently throughout the titration. This method cannot be used if the urine has been preserved with chloroform. If less than 5 cc. of urine is required for the titration, dilute the urine and repeat.

Calculation: 25 cc. of Benedict's solution are equivalent to .050 gm. of glucose. To obtain the percentage of sugar in the urine, divide .050 by the number of cc. of undiluted urine used and multiply by 100. A short cut is as follows: 5 divided by the number of cc. of undiluted urine gives the percentage directly.

Tubercle Bacilli.

Spin a test-tube full of urine at low speed for 2 minutes. Pour off the supernatant urine into another tube and spin this second tube at high speed for 50 minutes. Stain the second sediment for TB and examine.

Guaiac Test.

When a guaiac test is requested, perform it in the usual manner.

URINE ANALYSIS—*continued*

Benz-Jones.

Fill a thin-walled test-tube three quarters full of urine. Heat gradually in a water bath to about 55° C. Benz-Jones protein will disappear on cooling and reappear on heating. The protein is not stable and found only in fresh urine.

Culture.

Routine cultures are planted in tubes of plain bouillon and on plain agar slants.

Diazo Reaction.

Solution 1. (Sulphanilic acid, concentrated HCl, water.)

Solution 2. (.5% aqueous sodium nitrate.)

To 5 cc. of solution (1) add 3 drops of solution (2).

Add 5 cc. urine. Shake and add quickly 1 cc. ammonia water. Shake vigorously. A carmine color, particularly in the foam, denotes a positive test.

(4) RENAL FUNCTIONS

Draw up into a sterile 2 cc. syringe with a hypodermic needle an ampule of the standard phenolsulphonephthalein preparation. Expel the contents until exactly 1 cc. remains. This represents .006 of the drug. The patient voids just before or immediately following the injection. The drug is injected deep into the deltoid muscle. The patient drinks one to two glasses of water and the urine secreted during the next 2 hours and 10 minutes is collected. The specimen is sent to the laboratory, where it is diluted with tap water up to 1,000 cc. and made alkaline with concentrated KOH. The solution takes a red color. Using a test-tube of the same size compare the preparation with the standard. The normal excretion is above 35%. Observe if the sample was collected on time.

(5) METHOD OF CLEARING URINE OF BILE AND BLOOD FOR MORE ACCURATE ESTIMATION OF RENAL FUNCTIONAL TEST

Collect the renal function in the usual manner and make up to 500 cc. Mix well. Take 20 cc. of this and add 20 cc. of a saturated alcoholic solution of zinc acetate. Shake and filter. To 20 cc. of the filtrate, add 5 cc. of sodium hydroxide of any moderate strength. Add 15 cc. of water. Mix well and compare with the color scale. Multiply the reading by 2 for the final result.

(6) PROSTATIC SMEAR

Examination is best made with a full bladder. Massage the prostate and vesicles from above downward. Massage the lateral lobes with a firm rotary motion. Continue the massage until a discharge is obtained. When the secretion is in small amounts it can be more readily obtained by "milking" the penis after the massage. The secretion is collected on a glass slide stained with Gram's stain and examined under the oil immersion. Report the number of cells per oil field and describe any organisms.

(7) CATHETERIZATION

A sterile catheter, sterile catheter lubricant, hemostat, sterile sponges, one sterile towel, soap, and bichloride solutions are required.

Wash off the glans penis with soap, then bichloride solution, using sterile sponges. Spread the sterile towel across the upper thighs and wrap the shaft of the penis with a sterile sponge. Grasp the penis with the left hand. Lubricate the catheter tip and grasp the catheter about two inches from the end with sterile hemostat. Feed the catheter slowly into the urethra with the hemostat, not touching it with the hands except at the distal end.

It is not necessary to scrub up if this technique is followed.

(8) STOOLS

Amount.

While only the 24-hr. amount is of importance, the amount arriving at the laboratory is recorded.

1. Small. If solid, if not sufficient to cover the bottom of the usual stool jar. If fluid, filling the jar only 1 inch or less.
2. Large. Enough to fill the jar to 1 inch of the top.
3. Medium. Filling the jar more than 1 inch from the bottom and less than 1 inch from the top.

Consistency.

1. Fluid.
2. Soft unformed, without shape but cannot be poured.
3. Soft formed, with shape, but soft.
4. Hard formed, with shape and hard.
5. Partly formed, lumps floating in fluid.
6. Foamy.

Odor.

Only exceptional odors are recorded.

Color.

Only exceptional colors are recorded.

Mucus.

Seen on gross inspection. The surface is explored with a glass rod if the stool looks slimy. Any tenacious viscid material forming strings when teased out by the rod is considered mucus.

Pus.

Microscopic creamy material showing white blood cells on smear.

Blood.

Microscopic red blood. "Tarry" stools are not included.

STOOLS—*continued*

Fat.

If the stool looks greasy or is full of curds, or if fat is suspected or inquired for, a drop of Sudan III is added to a drop of diluted stool. If droplets or crystals take the stain in excess of normal, fats are increased. Normal stools should be frequently examined to control the observation.

Starch.

A drop of diluted stool is examined for starch granules, and, if none are seen, starch is reported as absent. If starch is particularly inquired for, a drop of Lugol's solution is added to a drop of diluted stool and anything taking the stain is reported as starch.

Muscle Fibers.

A drop of diluted stool is examined microscopically and yellowish more or less square-ended bodies showing transverse striations are reported.

Ova.

Gross: Routinely a simple inspection is enough. If a special parasite hunt is requested, the stool is washed through a sieve by a stream of water and the sediment examined.

Microscopic:

1. Routine examination:

(*a*) Emulsify 2 gm. of stool in water.

(*b*) Filter through two layers of gauze.

(*c*) Centrifuge and pour off supernatant cloudy fluid.

(*d*) Fill up tube with water, stir and centrifuge again.

(*e*) Repeat (*d*).

(*f*) Examine sediment for ova.

2. Method for hookworm:

(*a*) Emulsify 50 to 100 gm. of stool with saturated solution of NaCl in glass with straight sides.

STOOLS—*continued*

(*b*) Press pad of steel wool about ½ in. thick down to filter stool.

(*c*) Let stand 5 to 10 minutes.

(*d*) Examine surface fluid for ova.

Surface fluid best obtained by using a fine wire loop 1 cm. in diameter.

Guaiac Test.

To about 5 cc. of stool suspension, about 1 cc. of glacial acetic acid is added and stirred thoroughly. Add a few cc. of ether and stir again.

A pinch of gum guaiac is placed in a test-tube and the tube almost filled with 95% alcohol. This is shaken and allowed to stand for a few minutes. Then 1 to 3 cc. of hydrogen peroxide is added and mixed by inverting the tube several times with the thumb over the end. This solution is then overlaid on the acidified stool suspension. A blue ring indicates a positive test.

Microscopic.

A half teaspoonful of stool is placed in a wineglass and the glass two thirds filled with water. Stir thoroughly with a glass rod. Drops taken from this suspension are examined microscopically with a low power. High power is used only to identify a particular structure. No attempt is made to identify all the objects in the field; only starch, fats, muscle fibers, and parasites are searched for. After a drop has been removed for microscopic examination, the remainder of the contents of the wineglass is used for the Guaiac test.

Bile.

The color of the stool is the most important test for bile. This is observed routinely. If a special test is requested, 1 in. of fecal suspension is placed in a test-tube and 3 in. of saturated $HgCl_2$ solution is added and shaken. A pink color developing after twelve hours indicates bile.

STOOLS—*continued*

Tubercle Bacilli.

The patient should be prepared as follows: Thoroughly purge until only mucous stools result. Either a mucous stool may be used or material collected by means of a proctoscope. Select a small piece of mucus from the stool and make a thin, watery suspension. Centrifuge at lowest speed for 2 minutes. Pour off the supernatant liquid into another test-tube and centrifuge this at high speed for 30 minutes. Both sediments are then smeared and stained for acid fast bacilli.

Amœbæ.

Prepare the patient as above. Warm a cover-glass, cover-slip, and microscope to body temperature by placing them in an incubator. Obtain the stool or proctoscope scrapings and place immediately in a warm receptacle. Smear the material on the warm slide, cover with a cover-slip, and examine at once for amœbæ with low and high power. It is useless to search for amœbæ in a cold stool. Do not call objects amœbæ unless they show pseudopodic activity.

(9) ROUTINE BLOOD EXAMINATION

The red count and white count is done in the regulation manner.

Platelets. The method that we have found most satisfactory is using a diluting solution composed as follows:

Dissolve 6 gm. of glucose (dextrose pure Merck) and .05 gm. of sodium citrate (neutral) in 100 cc. water. Filter. To this add .02 gm. neutral red and shake until dissolved. Then add .4 gm. of crystal violet (pentamethylpararosanilinhydrochloride) and heat to 65°C., holding at that temperature for 5 minutes. Let cool to room temperature slowly with shaking; centrifuge at high speed; filter twice through two thicknesses No. 30 filter paper. Add 4 drops of formalin. A third filtration may be necessary.

ROUTINE BLOOD EXAMINATION—*continued*

Draw up the solution to the .5 mark of a red-cell counting pipette. Then blood from a fresh puncture wound of the ear to the 1 mark. Rapidly fill the red pipette with the solution to the mark 101. Shake vigorously. Count the platelets, using the high dry as in a red count. If desired, one can add a small quantity of cresyl blue to the solution, which stains the platelets a pale blue. The average platelet count with this technique is slightly lower than the figures given as normals (e.g. 200,000 to 350,000).

(10) RETICULATED ERYTHROCYTES

Slides with dried cresyl blue are obtained from the Medical Research Laboratory. A small drop of blood is placed on a cover-slip and inverted on the cresyl blue. The cover-slip is then drawn off the slide in such a manner as to give a thin smear. Stain the smear with Wright's stain and count the reticulated cells. One thousand red cells are counted.

(11) TECHNIQUE FOR COLLECTING BLOOD

Apparatus: Tray containing two receptacles (ordinary drinking glasses are satisfactory), a 10 cc. glass syringe, assorted needles immersed in alcohol, test-tube, labels, tourniquet, sterile sponges, collodion, a flask containing sterile salt solution with appropriate connection to fit the needles.

One receptacle is filled with sterile salt solution in which the syringe is kept when clean. Select a needle and wash it out with sterile salt solution from the flask for that purpose. Apply the tourniquet above the elbow. Have the patient clinch his fist tightly and scrub the site with a sterile sponge which has been moistened with alcohol. Obtain about 6 cc. of blood. Without removing the needle, expel the contents into a test-tube, replacing the cotton plug at once. The syringe is cleansed by repeatedly drawing up several cc. of sterile salt solution and expelling the contents into the other

TECHNIQUE FOR COLLECTING BLOOD—*continued*

(empty) glass. Finally cleanse the needle in a similar manner and replace it in the alcohol. The puncture wound is painted with collodion. If this technique is carried out, the blood is not hemolized and rarely infected.

(12) DIRECTIONS FOR TAKING BLOOD FOR CHEMICAL ANALYSIS

Chemical tests of blood are done exclusively on whole blood or plasma, therefore do not allow blood samples to clot. It is not necessary to keep samples absolutely sterile. Determination of sugar and carbon dioxide can be made only on fresh blood, therefore do not take blood for these determinations on Saturday afternoon, on Sunday, or on holidays. All samples should be placed in laboratory refrigerator as promptly as possible. Blood sugars should be taken fasting, most conveniently before breakfast. N.P.N.'s are also more accurate if taken fasting. Take samples only in special bottles which may be obtained in the chemical laboratory. These bottles are charged with the proper amount of oxalate or citrate.

Use Potassium Oxalate Bottles for:

Test	*Amount of Blood Required*
Non-protein Nitrogen	5 cc.
Urea	5 cc.
Uric Acid	10 cc.
Creatinin	5 cc.
Sugar	5 cc.
Carbon Dioxide	5 cc.

Use Sodium Citrate Bottles for:

Calcium	20 cc.
Chlorides	5 cc.

Use either Citrate or Oxalate Bottles for:

Phosphates	5 cc.
Fat	5 cc.
Hemoglobin (Palmer method)	5 cc.
Oxygen	10 cc.

(13) BLOOD CULTURES

Any method that avoids contamination is satisfactory. It is not necessary to scrub up. A satisfactory method is as follows:

Boil up a 30 cc. syringe on which has been placed a large, sharp needle. Gauge the size of the needle to be used according to the size of the patient's veins, using as large a needle as possible. Paint the skin with full strength (7%) tincture of iodine and then apply a sponge which has been saturated with alcohol. Enter the vein without touching with hand the skin where the needle pierces it. Obtain 30 cc. of blood. Remove the needle from syringe by grasping it with a sterile sponge. Expel a few drops of blood, flame the point of the syringe, and expel 25 cc. into a flask of bouillon, the edge of which has been flamed by a nurse. The nurse flames the edge of the flask again and replaces the sterile plug. Expel a few more drops, reflame the end of the syringe, and place the remaining 5 cc. of blood in the second flask as before. The flasks of bouillon are obtained from the Pathological Laboratory and should be placed in the incubator before using until warmed. Use them at once and replace as soon as possible. Great care must be taken to avoid contamination. The nurse should be thoroughly instructed and the procedure done as quickly as possible. If more than one flask in ten is contaminated it indicates negligence.

(14) BLEEDING TIME

Bleeding time is calculated by the duration of the hemorrhage. The formation of the crust is immaterial. A deep puncture wound is made with a Glover's needle into the lower lobe of the ear. At intervals of 30 seconds remove the blood with a piece of filter paper. As there is a great deal of variation in this test as to the depth of puncture wound, size of the needle and pressure that is used to remove the blood, it is well to observe one of your superiors do it before attempting it yourself. Normal bleeding time is less than 6 minutes.

(15) COAGULATION TIME

Apparatus: A rack containing small test-tubes 1x7 cm. which are perfectly clean and dry. Rinse the tubes with sterile salt solution. Rinse the fifth tube with calcium chloride solution. A 10 cc. syringe is thoroughly cleansed with water and alcohol and dried with ether. The needle is similarly prepared. Obtain by venous puncture 8 cc. of blood. The puncture should be done with as little trauma as possible. One cc. of blood is transferred as neatly as possible (after the needle has been removed) to each test-tube. The clotting time is calculated from the time of the puncture. After 2 minutes the first tube is tilted and this repeated every minute following until the tube can be inverted without disturbing the blood. The tube should be agitated gently at the end to be certain that the clotting is complete. Rough agitation may permit defibrination. Surface coagulation may appear at normal time in 6 to 12 minutes, but it may take much longer for a firm clot to form throughout. This should be noted. After the first tube has firmly clotted, the second may then be tilted in which the blood usually will not have formed as firm a clot. Tilting should be done regularly until the blood is firm, the clotting time ending where the next tube to be tilted is found to contain a firm clot. This may be the third tube, but it is usually the fourth. The tube containing calcium chloride should be tilted separately from the other four in the same manner, the difference in coagulation time being noted. Place the rack in the incubator for 24 hours to observe the presence of a fibrolysin ferment and clot retraction.

(16) CLOT RETRACTION

Either the third or fourth tube (mentioned under coagulation time), which has not been much disturbed while undergoing the clotting process, should be placed in an incubator at 37½°C. and observed after 12 to 24 hours. The clot retraction will not ordinarily occur if the blood has been considerably agitated while clotting. Normally a definite retraction may be observed in 6 hours but the process is often not complete for 18 hours. The color and firmness of the clot should also be observed.

(17) THE FIBROLYSIN TEST

The presence of a fibrin dissolving ferment may be determined by observing whether the clot undergoes complete or partial dissolution within 18 hours, when the blood is kept sterile at body temperature. A normal clot will show no such dissolution in this time, while some abnormal bloods may show complete dissolution even in 6 hours. (Reference: Drs. Lee and Minot, in "Nelson's System," Vol. 4, page 111.)

(18) BLOOD GROUPING

A direct and an indirect grouping must be performed on both donor and recipient. Stock sera are kept in Medical Research Laboratories.

Indirect Grouping.

Make a suspension of cells in a small test-tube by adding 3 or 4 drops of blood obtained from the ear to 3 cc. of 1½% sodium citrate solution. Place 1 drop of this suspension on each end of a clean glass slide. To the suspension add 1 drop of Group II serum, and to the other, 1 drop of Group III serum. On a second slide add 1 drop of the suspension and with clean cover-slips mix the materials and then cover. Examine under low power. Agglutination is usually very rapid and very definite. The cover-glass should always be raised and the cells and serum remixed several times before a negative reading is made. It is wise to allow the mixture of cells and serum to remain in contact for at least 30 minutes, preferably in an incubator, before reporting negative agglutination. If such time is allowed, drying must be prevented by sealing the edges of the cover-slip with melted vaseline.

If the reaction is doubtful, it is possible by employing different amounts of cells and serum (by incubating the mixture for some hours), and by thoroughly

BLOOD GROUPING —*continued*

washing the red cells, to decide the problem of doubtful reaction. A safe rule is never to regard as negative a reaction in which there is any doubt. Distinguish rouleaux formation from true agglutination. Confusion may be caused by atypical agglutinations and allied phenomena which are little understood. The second slide is used as a control.

By reference to the table below, determine the group of the blood. If Group II, III, or IV, check against the respective serum:

		Serum			
		1	**2**	**3**	**4**
Cells	**1**	0	+	+	+
	2	0	0	+	+
	3	0	+	0	+
	4	0	0	0	0

0 = No agglutination + = Agglutination

Group I "universal recipient" will take any group.

Group IV "universal donor" can be given to all groups but can only take a IV.

Groups II and III will take their respective groups or a IV.

Direct Grouping.

Obtain several cc. of the patient's blood in a small test-tube and allow to clot. Make a suspension of the donor's corpuscles as above. Place on a slide 1 drop of the suspension. Add 1 drop of the recipient's serum and determine the presence of agglutination.

(19) FRAGILITY OF THE ERYTHROCYTES

Five cc. of fresh blood is placed in an approximately equal volume of isotonic salt solution and citrate (1½% citrate solution in .9% salt solution). This is placed in a graduated centrifuge tube, the corpuscles spun down, and the supernatant fluid pipetted off. The corpuscles are then washed once with isotonic salt and diluted to form a 1:5 suspension. Into each of 16 to 18 small test-tubes (1–7 cm.) is pipetted 1 cc. of the stock salt solutions (from .52% to .18%). These stock bottles are found in the blood laboratory. To each tube add 1 drop of the corpuscle suspension. Each tube is inverted once with the finger over the end, the finger being wiped on a cloth between each inversion. The rack is placed on ice and read about 12 to 18 hours later. The tube in which hemolysis begins and the tube in which it is complete (no corpuscles remaining on the bottom) are recorded (e.g. hemolysis begins at .44% and is complete at .28%).

(20) PLASMA DILUTION

Blood is collected in a small, clean test-tube in a small amount of potassium oxalate solution (.1 of a cc. of a 10% solution will suffice for 10 cc. of blood; this is the optimum amount). Invert the tube several times; do not shake vigorously as hemolysis spoils the test. Centrifuge at high speed.

Dilution of the plasma is carried out as follows: Take 5 drops of oxalated plasma and add 25 drops of distilled water, giving a dilution of 1:6. Compare this to an equal quantity of distilled water in a tube of the same size, gauging the color by looking down on top of the tube. Subsequent dilutions may be made on the diluted plasma, always maintaining the quantity at 30 drops. The end point is reached when only the faintest discoloration remains as compared to the tube of distilled water.

A plasma must always be clear. Fat or hemolysis renders the test unreliable. It is well to collect the blood when the patient is fasting. The pipette should be gauged so that 30 drops equal approximately 1 cm. Normal serum color

PLASMA DILUTION —*continued*

usually disappears in dilutions of less than 1:30, in anemias it may run as high as 1:70, and in jaundice cases it is often over 1:300.

(21) TRANSFUSION OF BLOOD

"Medical" Method

Apparatus: Sterile dry goods, seven 30 cc. syringes, sterile tourniquet, sterile Vincent needles with short tubing to fit the syringes, sphygmomanometer, and solutions (1½% citrate solution, cold Ringer's solution and warm Ringer's solution).

A catalogue of donors is kept in the Director's office. Each donor must be indirectly grouped, have a negative Wassermann reaction and a negative venereal history. The genitals and skin are examined before the transfusion to detect a recent infection. The patient is then directly grouped with the donor (patient's serum against donor's corpuscles). The patient's red count and hemoglobin is taken before the transfusion. A light diet is given the day of the operation.

The patient and donor are placed on trucks which are so arranged that the operators may stand between the trucks without crowding and the best veins are most accessible. The trucks are draped with sterile linen, while the patient holds the arm upright. The entire arm is then surgically cleansed and draped.

The sphygmomanometer is placed on the donor's arm with the bulb and gauge placed in such a manner that a nurse may manipulate it without contaminating the field. The sterile tourniquet is used on the recipient. Before proceeding further it is best to practise the technique so that each person will understand exactly what he or she is to do. The syringes after being rinsed successively with cold Ringer's solution, hot Ringer's solution, and finally in the citrate solution are placed within easy reach of the operator who is to obtain the blood from the donor. He then fills the syringe with as little manipulation as possible, calling off the number of cc.'s in units of 5 as a guide to the other operator who is

TRANSFUSION OF BLOOD — *continued*

simultaneously giving the preceding syringe full to the recipient. When 30 cc. are obtained he removes the filled syringe with his right hand, compressing the tube with his thumb and index finger of the left hand. The syringe is then passed to the other operator who takes it with his right hand and connects it with the needle and starts the injection slowly. The donor's operator then attaches another syringe and repeats the process until the desired amount of blood is obtained. The recipient's operator places his empty syringes at the foot of the truck. The nurse cleans them as described above and replaces them on the donor's truck.

On each truck a syringe of warm Ringer's solution is kept ready to flush out the needles if necessary and to be used at all times when the operation is held up for any reason.

A constant flow either in or out must be maintained; otherwise the needle will become plugged. When every one is familiar with the process, the sterile tourniquet is applied to the recipient's arm and the sphygmomanometer on the donor's arm is inflated to diastolic pressure. Everything is now ready to start. The operator having the poorer vein enters first and injects salt solution slowly until the other operator is ready. The procedure described above is then carried out. Between syringes the sphygmomanometer, which is maintained at diastolic pressure while the blood is being withdrawn, is deflated to allow the blood to flow with greater freedom into the forearm. It is often of assistance to have the donor slowly close and open the hand when the blood is being withdrawn. The syringe count is kept by the person who manipulates the sphygmomanometer.

Six hundred cc. is the usual transfusion. It should be completed in 6 to 8 minutes. When the patient arrives on the ward, repeat the red count and hemoglobin.

(22) FORMULA FOR BLOOD VOLUME AFTER TRANSFUSION

$$\text{Volume of Transfusion} = \frac{\text{(Donor's count)} - \text{(Patient's count after)}}{\text{(Patient's count after)} - \text{(Patient's count before)}}$$

(23) THERAPEUTIC BLEEDING

A liter flask is fitted with a suction apparatus. A large needle is inserted into a vein, the suction apparatus applied, and the desired amount of blood withdrawn. Collect the blood aseptically and deliver to the Medical Research Laboratory.

If the case is an urgent one and the patient restless, it may be better to cut down on a vein. An incision is made just below the bend of the elbow and the vein dissected free. A ligature is then placed above and below, but not tied. These ligatures are used to stop the flow of blood by traction. A sphygmomanometer is applied, and inflated to approximately diastolic pressure. Nick the vein with scissors and bleed slowly to the desired amount. It is well to take frequent blood pressure readings from the other arm. Tie the vein off and close the wound with a continuous suture.

If there is any doubt of the diagnosis do a red count before starting.

SPUTUM

(24) EXAMINATION OF SPUTUM FOR ELASTIC FIBERS

Procure at least two or three boxes full of sputum if possible. Add an equal quantity of 10% sodium hydroxide. Boil slowly with frequent stirring until a homogeneous mixture is obtained. Centrifuge the entire quantity in as many tubes as necessary and pour the sediments together with a small amount of supernatant liquid. Centrifuge at high speed. Examine the sediment carefully by means of the low and high power. If a suggestive fiber is seen, stain with an elastic tissue stain. It is the experience of beginners to err on the positive side. If in any doubt, consult one of the staff physicians.

(25) SMITH'S STAIN FOR SPUTUM

A large, clean cover-slip is held by means of a holder and a very thin smear is made from a selected portion of the sputum. Dry and fix by passing rapidly through a flame. Great care should be taken not to char the smear. This is best done by making two or three rapid insertions through the middle of the flame. This stain cannot be done properly until one becomes thoroughly accustomed to the staining properties of the organisms and cells and the strength of the various solutions. The decolorizing in this stain is very important.

With the preparations which we ordinarily have on the wards, the technique is approximately as follows:

Cover the smear with aniline oil gentian violet. Pass quickly through the flame once and wash off with Gram's iodine solution, leaving the smear covered with this solution. Pass through the flame quickly twice and decolorize with 95% alcohol, washing off both sides of the cover-slip. This is usually done by allowing the alcohol bottle to touch the edge of the cover-slip, which is held vertically. Only the faintest traces of blue color should remain. Wash off the alcohol by running the cover-slip with the smear side down through a gentle stream of water. Add Smith's eosin (solution may need dilution with water) and pass through the flame quickly. Wash as before under a gentle stream of water. Add Loeffler's methylene blue. Stain about 10 seconds. Wash with water.

The smear is now dehydrated, first by washing with 95% alcohol, then with absolute alcohol, and finally with xylol. Place the cover-slip smear side downwards upon a drop of Canada Balsam which has been placed upon a clean slide. The xylol will dissolve the balsam, and a clean, dry preparation can be made by pressing a towel or gauze firmly down on the cover-slip. Examine with the oil immersion. Gram positive organisms are stained a deep purple, Gram negative organisms a light blue, the cytoplasm of the cells a light pink, and the nuclei a deep purple. The stroma takes a light blue stain.

(26) PREPARATION OF CERTAIN STOCK SOLUTIONS

Sodium Bicarbonate.

Bring a liter of sterile freshly distilled water to the boiling point. Add immediately 30 gm. of sodium bicarbonate (C.P.) taken directly from the original container and weighed in a sterile vessel. Cool the solution to the desired temperature and use at once. Sodium bicarbonate solution cannot be sterilized by boiling, on account of the transformation of bicarbonate into the carbonate.

Dextrose Solution.

A 5% dextrose solution in salt solution is sterilized according to the usual technique and injected intravenously up to 500 cc. The injection must be made very slowly, taking at least 30 minutes for 500 cc. Salt solution is given before and after the glucose. This solution is ordinarily kept prepared in the amphitheater. Do not use a cold preparation.

(27) GASTRIC ANALYSIS

Gastric contents should be obtained as early as possible in the morning in order to allow the patient his breakfast. The contents need not be titrated until later. The patient is seated upright in a chair and draped with rubber apron, sheet, etc., provided by the nurse. The procedure may be done in bed if the patient is too sick to sit in a chair. Careful instruction should first be given the patient as to what is to be done and the reason for the analysis, to gain his cooperation. It is well to tell the patient that he may choke a little at the beginning. Instruct him how to breathe, and assure him that as soon as the tube passes the throat he will be comfortable.

A Rehfuss tube that has been previously chilled with ice is used. Stand to the patient's right and slightly behind him. Throw the head back, have the patient open his mouth widely and place the tip of the tube on the posterior part of the tongue. When the patient says "Ah," quickly force the tube into the posterior pharynx and the first convulsive

GASTRIC ANALYSIS—*continued*

swallow usually carries it past the thyroid cartilage. With the fingers near the patient's lips, continually feed the tube into the mouth. The tube should never be pushed into the mouth in excess, but there should be enough slack in the mouth for the patient to swallow on. Then instruct the patient to breathe deeply through the mouth, swallowing occasionally. The tube usually passes down readily. Retching is sometimes experienced when the tube is in the neighborhood of the cardia. A few extra swallows and the tube is in the stomach. Some patients are able to take the tube more easily if left entirely to themselves, and this method should always be tried in case the regular procedure fails.

The tube is marked at 40 cm. (cardia), at 55–60 cm. (duodenum). Insert the tube about 50 cm. The fasting contents is then withdrawn by means of the syringe. Insert air to clean out the tube and to balloon out the stomach, if it is desired to percuss its position. One should always be able to obtain at least 10 cc. of fasting contents. If the patient salivates a good deal, he should be instructed not to swallow but to use gauze or a basin.

The test meal is then given (one slice of bread without crust and a full glass of water, the patient being instructed to take it all and not drink the water up first). Forty-five minutes later the test meal is withdrawn. In withdrawing the contents care should be taken to shift the level of the tip several times before removing the tube to be sure all the contents have been evacuated.

Examination.

Total amount, gross appearance, color, odor, and amount of sediment should be recorded. A drop or two of each should be examined microscopically by placing a few drops of the material on a slide and adding a drop of Lugol's solution and covering with a cover-slip. The usual appearance of the fasting contents is a few epithelial cells, white blood cells, mucus, and occasionally Oppler-Boas bacilli or sarcinæ. The usual pathological finding consists of an excess of white cells, the presence of red cells and residual food,

GASTRIC ANALYSIS—*continued*

which is usually present in the form of starch and takes the Lugol's stain. Several drops are taken from different levels of the contents. Do a guaiac test on both the fasting contents and the test meal.

Acidity.

Pipette 10 cc. of the fasting contents into an Ehrlenmeyer flask. Add an equal quantity of distilled water and shake until homogeneous. Add three drops of Töpfer's reagent (Dimethylamidoazobenzol). In the presence of free HCl a carmine red color is present. If no free HCl is present the fluid turns a canary yellow. Titrate the material against decinormal sodium hydroxide. The end point is the disappearance of the red color. Note the number of cc. of sodium hydroxide used and multiply by 10. This indicates the amount of free acid present in 100 cc. of gastric contents. Add a few drops of phenolsulphonephthalein indicator and continue the titration until the first permanent pink color appears throughout the flask. Note the total number of cc. of sodium hydroxide used, multiply by 10, and the result is the total acidity in 100 cc. of gastric contents. Repeat in a similar way, using the test meal.

Lactic Acid.

In the absence of either a free or combined acid it is routine to determine the presence or absence of lactic acid in the following manner:

> In a test-tube half full of water add 5 to 10 drops of 10% ferric chloride solution. Continue to dilute this until the faintest trace of yellow remains. With about 10 cc. of this in each of two test-tubes, add a few drops of the gastric contents to one tube, and if the color darkens perceptibly it denotes the presence of lactic acid.

(28) STOMACH WASH

Prepare the patient with a rubber sheet, a large basin, etc. Use a large rubber tube with bulb and funnel attached. The tube is pushed down the gullet to the mark on the tube. If trouble is experienced in passing the tube, have the patient lean forward as far as possible. This position renders the gullet almost horizontal. Stand to the patient's right and slightly behind, and with the left hand around the patient's shoulder push the tube into the gullet with the right hand, guiding with the left. The tube can be passed in a few seconds. After the tube is in the stomach the patient may sit upright.

The irrigating solution (usually water), which has been heated to slightly above body temperature, is poured down the funnel until the stomach is filled. The tube is then inverted over the basin and either siphoned out or drawn out by means of the bulb. If the water is not readily siphoned the patient should be instructed to tighten the abdominal muscles by "straining." This procedure is repeated several times and in a case of poisoning or alcoholism should be continued until the water returns perfectly clear.

(29) EXAMINATION OF SPECIMENS OF PUS

Make a thin smear and do a Gram stain, reporting the organisms found. If indicated, plant on appropriate media. Record also odor and gross appearance.

(30) SCHICK TEST

Usually a small outfit comes ready to make up a fresh solution of toxin and heated toxin. Take the glass capillary tube marked "Toxin" (not heated). Break off both ends of this tube and blow the contents into the Bottle "A," 10 cc. sterile salt solution. Now take the capillary tube of heated toxin and blow this into Bottle "B" of 10 cc. sterile salt solution. The solutions are now ready for use. Take .2 cc. of toxin solution from Bottle "A" and make a wheal on the flexor surface of the forearm. Be sure that this is injected

SCHICK TEST—*continued*

intracutaneously. Do the same with heated toxin solution from Bottle "B." The toxin or first wheal should preferably be the upper one just below the bend of the elbow and the control wheal several cm. below this. A tuberculin syringe with a fine intracutaneous needle is best suited for this work.

(31) TEST FOR SENSITIZATION TO HORSE SERUM

Cleanse the skin with alcohol. Allow the alcohol to dry. Inject with a small needle .2 cc. of sterile horse serum diluted 1–10 with sterile salt solution. The injection should be made intracutaneously, usually on the flexor surface of the forearm. A similar control wheal is made with salt solution alone. In five to ten minutes a wheal with a surrounding zone of erythema appears if the test is positive. The erythematous area may reach the size of a half dollar in positive cases.

(32) DESENSITIZATION OF A PATIENT TO HORSE SERUM

Before carrying out the sensitization test or the injection of serum, all patients should be questioned as regards a history of hay fever, asthma, or previous serum treatment.

As routine on all intravenous serum treatments .5 to 1 cc. of horse serum is injected subcutaneously several hours before the administration of serum. This will take care of most cases, even those with moderate sensitization.

In cases showing a marked reaction to the sensitization tests and also giving a history of previous difficulty, the following method is a safer one to use. Either this method should be used in such cases, or serum had best be omitted. The method is as follows: Inject .025 cc. of horse serum subcutaneously. Double this dose every half hour. If no reaction occurs from 1 cc. administered in this manner, then start with .1 cc. serum intravenously. Double this every half hour up to 25 cc., wait 4 hours and give 50 cc. of horse serum intravenously. Then after 6 hours proceed with the regular dosage.

(33) ABDOMINAL PARACENTESIS

Apparatus: Hypodermic syringe with 1% novocaine, paracentesis needle fitted with a rubber tube, rubber apron, and sterile dry goods. If possible, seat the patient upon the side of the bed or in a chair. Be sure the patient has voided or has been catheterized. If the patient is unable to sit upright, bolster him up as far as possible in bed by means of pillows.

Usually a low midline site is selected. Surgically cleanse the skin, cocainize the site, but not the deep tissues, as there is danger of the sharp needle penetrating the peritoneum and puncturing the gut. If a large paracentesis needle is used, it is well to nick the skin with a sharp scalpel. Grasp the needle near its point and introduce slowly in such a manner that it will not snap through the peritoneum. This is best controlled by grasping the needle within half an inch of the abdominal wall.

When the needle has entered the abdominal cavity, connect the sterile tubing and collect a small quantity for culture and pig inoculation. The remainder of the fluid is collected in a large receptacle. It is usually best to drain as much fluid as possible, and often it is necessary to compress and massage the abdominal wall. When most of the fluid is withdrawn, a loop of intestine frequently stops the flow. Then shift the needle slightly and massage the abdomen. When the drainage is complete, apply a thick sterile dressing, as there is usually considerable oozing, and an abdominal binder.

The fluid is examined for gross characteristics, cell count, differential count, cultural studies, search for bacteria, specific gravity, and quantitative albumen.

(34) ADMINISTRATION OF TETANUS ANTITOXIN

A prophylactic dose of 1,500 units U.S.A. may be given into the buttock by the method described for Gray Oil. The following plan is suggested for early acute tetanus by the "Tetanus Committee":

ADMINISTRATION OF TETANUS ANTITOXIN —*continued*

	Subcutaneous	*Intramuscular*	*Intraspinal*
1st day		8,000 units	16,000 units
2d "		8,000 "	16,000 "
3d "		4,000 "	4,000 "
4th "		4,000 "	
5th "	2,000 units	4,000 "	
7th "	2,000 "	4,000 "	
9th "	2,000 "	4,000 "	

The intraspinal administration is carried out in practically the same manner as outlined under 46 "Intraspinous Treatment." More spinal fluid should always be withdrawn than serum introduced.

Treatment of Acute Tetanus with Intraspinous Injection of Magnesium Sulphate

This method is used only in acute, severe cases. Etherize the patient and inject intraspinously 1 cc. of a sterile 25% solution of magnesium sulphate to every 20 lbs. of body weight. The patient should be propped up on a back rest after the injection. If sudden respiratory paralysis occurs, wash out the subarachnoid space with warm Ringer's solution and institute artificial respiration, if indicated, as long as necessary.

(35) PNEUMOCOCCUS TYPING

A. Mousing the Sputum.

If possible the patient should expectorate directly into the sterile container. This is usually impossible and fresh sputum will do. A smear of the sputum is first made and examined. Select a bean-sized portion of the sputum and place it in a sterile Petri dish. Add about 2 cc. of sterile salt solution. Emulsify the sputum by sucking it in and out of a sterile 2 cc. syringe. Wash off the mouse's belly with alcohol and inject 1 cc. of the sputum suspension into the peritoneal cavity, using a blunt-pointed needle to avoid injury to the viscera.

PNEUMOCOCCUS TYPING — *continued*

B. The remainder of this procedure is performed by House Officers only when the technician is not available.

The average time for sufficient growth of the pneumococci is 6 to 8 hours. If there is any doubt (that is, if the mouse does not look very sick) the peritoneum should be punctured with a small hypodermic needle and syringe and a drop of exudate obtained, smeared, and stained. Fifteen pneumococci per oil immersion field are usually sufficient to proceed, although more are desirable.

If the mouse is not already dead, chloroform him in a small glass jar. Pin the mouse on his back to a board by four thumb tacks through the feet and expose the peritoneum aseptically. Puncture the peritoneum with a sterile blunt needle and wash out the peritoneal cavity with 4 to 5 cc. of sterile salt solution with a sterile medicine dropper, collecting the washings in a sterile centrifuge tube. Also plant a culture on a blood agar plate. Centrifuge the peritoneal washings at low speed (three notches) a few minutes. Decant the supernatant bacterial suspension into a second centrifuge tube and spin at high speed for 20 to 30 minutes (seven to nine notches). Pipette off clear fluid for the precipitin test. Add sufficient sterile salt solution to the bacteria in the bottom of the tube to make a moderately heavy suspension. A small rack is set up with nine small precipitin tubes, — four in one group and four in the other group, with one tube left for the bile soluble test.

Set up these tubes as follows:

GROUP A

I. .2 cc. serum type 1 (1–10 dilution) plus .2 cc. bacterial suspension.

II. .2 cc. serum type 2 (undiluted) plus .2 cc. bacterial suspension.

III. .2 cc. serum type 2 (1–10 dilution) plus .2 cc. bacterial suspension.

IV. .2 cc. serum type 3 (1–10 dilution) plus .2 cc. bacterial suspension.

V. .5 cc. sterile ox bile plus .2 cc. bacterial suspension. These should be shaken up.

PNEUMOCOCCUS TYPING—*continued*

GROUP B

I. .2 cc. serum type 1 (1–10 dilution) overlaid with .2 cc. of supernatant fluid.
II. .2 cc. serum type 2 (undiluted) overlaid with .2 cc. of supernatant fluid.
III. .2 cc. serum type 2 (1–10 dilution) overlaid with .2 cc. of supernatant fluid.
IV. .2 cc. of serum type 3 (1–10 dilution) overlaid with .2 cc. supernatant fluid.

These must not be shaken.

Incubate the above set-up for 1 hour and read, looking for macroscopic agglutination of bacterial suspension in Group A and a ring zone of junction in Group B.

(36) THROAT CULTURES

Culture tubes containing sterile swabs are found on the wards. Obtain a good view of the throat and tonsils by using a light and tongue depressor. Rub the swab against the tonsils and posterior pharyngeal wall, particularly any suspicious areas, and plant on blood serum. Label and place in the incubator. A nasal culture is obtained in the same manner by means of a nasal speculum. This refers to septum and definite suspicious areas in nasal chambers.

For naso-pharyngeal cultures introduce swab at anterior nares and force it gently back until obstruction of the naso-pharynx is met. This should be done with the aid of a speculum.

(37) THORACIC PARACENTESIS

A. For Diagnosis.

Boil up a 2 cc. syringe with small and long needle, and a 30 cc. syringe with a large, long needle. Select the place for tapping, generally in the 6th, 7th, or 8th spaces. Have the patient lean forward on a rest with the arms well out in

THORACIC PARACENTESIS—*continued*

front of him. If he is too sick for this, place the patient on the side opposite the one to be tapped, with a pillow under his chest to spread the ribs on the upper side. Cocainize the skin and pleura, allowing several minutes for the anesthetic to take effect. Two per cent novocaine or ½% cocaine is ordinarily used. One to two cc. should be sufficient. Insert the large needle on the end of a 30 cc. syringe, and keeping close to the upper border of the lower rib insert the needle slowly into the thoracic cavity. If no fluid is obtained, try again in various directions and at various depths. Thirty cc. of fluid should be withdrawn. After the puncture is completed, place the patient back in a resting position. It is well to have on hand 3 gr. of caffein to be injected intramuscularly in case of sudden respiratory distress, also have morphia ready. It is often desirable to give morphia before tap.

The following laboratory work is done on the fluid:

Cell Count.

This must be done immediately or the cells will settle out or become fixed in the pellicle.

Specific Gravity.

This also must be done at once, before the formation of a pellicle.

Smears.

A few loopfuls are placed on a cover-glass or slide and allowed to dry or dried by gentle heating. One is stained with Wright's stain and a differential count made. Another is stained with Gram's and a search for organisms made. In serous fluids with a few cells it is well to centrifuge first and use sediment for smears.

Culture.

On bouillon and agar.

THORACIC PARACENTESIS — *continued*

A Tubercle Bacilli search is made on a centrifuged specimen.

Pig Inoculation.

Several cc. of the sterile material are placed in a sterile test-tube and delivered to the Pathological Laboratory.

Esbach.

Dilute the fluid 1:5 or 1:10. Fill the Esbach albuminometer to the mark "U." Add Esbach's reagent to the mark "R." Invert gently twelve times. Allow to stand over night and read.

Gross description and amount of fluid obtained.

B. For Evacuation of Fluid.

Boil up the same apparatus but add the special chest tap needle or a lumbar puncture needle. Also boil up a three-way stopcock and tubing. Prepare the patient as before and insert the large needle. Attach the tube leading to one arm of the three-way stopcock to the needle, the 30 cc. syringe to a second tube, and the third tube is placed in a large bottle. Pump the fluid from the chest into the bottle. Save 30 cc. of the fluid for culture and tests as above. Take out all fluid that can be obtained and record the amount. In very sick cases and cardiacs, withdraw the fluid very slowly. When the patient coughs, it is usually a sign of the lung coming down on the needle and it is best to wait a few moments before proceeding again, or if the coughing persists to withdraw completely. Have ready a hypodermic of morphia and of caffein. Always bear in mind that sudden collapse may occur, and the patient should be aware of this possibility. The danger, of course, is very slight, and it is usually expressed to them by stating that they are running about as much of a chance as "taking a train out of the North Station."

(38) LUMBAR PUNCTURE

Place the patient on the bed or table in such a manner that his spine is horizontal with the edge of the table. The thighs are sharply flexed on the abdomen; the hands clasp the knees lightly; flex the head in order to obtain the maximum bowing of the spine. It is well to reassure the patient at this point and to encourage him not to alter his position. The lower back is then surgically cleansed and draped appropriately with sterile towels.

Using the iliac crest as a guide, locate the spinous processes and infiltrate the skin with 1% novocaine. It is not necessary to inject the anesthetic deeply into the tissues. With the thumb on the spinous process thrust the needle quickly through the skin midway between the processes, either in the midline or slightly to one side. Slowly introduce the needle with its point directed slightly upward. A slight sense of obstruction is met just before the needle enters the canal, and the dura usually but not always "snaps" as it is pierced.

If bony obstruction is met, partially withdraw the needle and proceed again with the point elevated a half centimeter. If again unsuccessful, repeat with the needle directed slightly downward. It is better to withdraw the needle completely if this procedure has also proved fruitless and try again in another space. "Dry taps" are not recognized. Do not try more than twice without the permission and guidance of a more experienced operator.

Examination of Fluid.

Pressure: Apply the manometer as quickly as possible. Observe the pulse and respiratory oscillations as well as the effect of coughing. After taking the initial reading, compress the jugular veins. The fluid should rise promptly if the hydrodynamics are normal. Withdraw 5 cc. by inverting the manometer and take a second reading. Withdraw a second 5 cc. and take the third reading. Remove the apparatus without disconnecting the manometer and dress the puncture wound with collodion. Place the patient flat on his back. No pillows are permissible for 6 hours.

LUMBAR PUNCTURE—*continued*

Color and Turbidity.

Observe any abnormal color and turbidity. In doubtful cases compare with tap water.

Cell Count.

Draw up into a white counting pipette glacial acetic acid to the point .3 mark and spinal fluid to the 11 mark. Agitate. This procedure dissolves the erythrocytes and the dilution may be disregarded. Either an ordinary counting chamber may be used in which ten large squares are counted, or a special counting chamber (2/10 mm. deep, i.e. twice usual depth) in which five large squares are counted. Repeat the procedure without using acetic acid. If there is any discrepancy between the two counts, it is fair to assume the presence of red cells in the undiluted fluid. If there were many red cells present, the cell count is of doubtful value. The fluid should be centrifugalized and the sediment stained with Wright's stain. Make a differential count and describe any abnormal cells. This procedure is not necessary if the cell count is less than thirty.

Protein Tests.

Five per cent sulpho-salycilic acid or alcohol tests are used routinely. Into a small test tube (1x7 cm.) equal portions of the reagent and fluid are placed. Mix by inverting several times. If there is more than the faintest cloud in the alcohol test, it indicates an increased protein content. The sulpho-salycilic acid test is more delicate and a slightly greater cloud is normal.

Globulin Test.

Place .5 cc. of fluid in a small test-tube and underlay with an approximately equal amount of saturated solution of ammonium sulphate. To insure a saturated solution add a drachm of crystalline ammonium sulphate to a four-ounce stock bottle. Allow to stand 5 minutes. A cloud at the junction of the two fluids indicates the presence of globulin.

LUMBAR PUNCTURE—*continued*

The remainder of the fluid is put in the Wassermann ice chest for the Wassermann reaction, total protein, etc. If a gold solution test is desired, label the tube accordingly. If a sugar determination is wanted, place 2 cc. in separate tube, preserve with one drop of 10% formalin and place in the chemical ice chest. Always save one sterile tube.

Examination for Tubercle Bacilli.

Centrifuge all available fluid at high speed for 1 hour. Pour the supernatant fluid until about 5 cc. remain. Place this in a small test-tube. Add enough alcohol to bring out a definite cloudy layer. Centrifuge at a high speed for 30 minutes. Stain the precipitate and examine for tubercle bacilli.

(39) CISTERN PUNCTURE

The patient assumes a position as for a lumbar puncture. The same apparatus is used. The back of the neck up to the occiput is shaved and surgically cleansed. The head rests on a small pillow so that the whole spine is in the same horizontal plane. Draw an imaginary plane from the glabella through the superior portion of the auditory meatus and where this line strikes the back of the neck in the midline is the average site for the insertion of the needle. By deep palpation the spine of the second cervical vertebra is located, the skin cocainized, and a needle inserted just above the spine. Introduce the needle slowly and with great care in the direction of the same imaginary line spoken of above. It is better to tilt the needle slightly above this line at first until it strikes the base of the occiput and then to tilt the needle downward slightly and proceed cautiously until fluid is obtained. The average distance is 4 to 5 cm. but it is safer to withdraw the stylet every half cm. from 3½ cm. onward.

(40) PERICARDIAL PARACENTESIS

Apparatus: That used for an exploratory chest tap. The patient lies flat on the back to allow the heart to drop away from the chest wall. There is no real point of election. The tap may be performed at the area of greatest bulging, just outside the nipple line in the fourth space, or at the sternal xyphoid junction. If the last site is chosen, the needle is directed upward and to the left. Infiltrate the skin with 1% novocaine and proceed as in a chest tap, using, of course, greater precautions. Make a routine examination of the fluid obtained. (See Thoracic Paracentesis.)

(41) SUBPECTORAL INJECTIONS

The apparatus and solution are obtained from the amphitheater. It is a rule that the technique should be demonstrated by one of the upper men. Have ready for him a 2 cc. syringe, 1% novocaine, sterile sponges, iodine, and collodion.

(42) PREPARATION OF ARSPHENAMIN

Apparatus Needed: A flask of freshly distilled water, an empty sterile flask, sterile filter paper, sterile funnel, litmus paper, arsphenamin ampule, small file, and a small bottle of 15% sodium hydroxide.

Concentration: The solution is made up in a concentration of .1 gm. arsphenamin to 20 cc. of freshly distilled water. If a considerable quantity is to be given several hundred cc. may be made up at once. Put the number of cc. of distilled water into the sterile flask to correspond to the amount of arsphenamin to be dissolved. (For example, 120 cc. of distilled water for .6 gm. arsphenamin.) Now make a nick in the neck of the ampule with the file. Knock off the glass neck of the ampule and empty contents into the arsphenamin flask by tapping on the base of the ampule with the file if necessary. Rotate the flask gently to dissolve the

PREPARATION OF ARSPHENAMIN—*continued*

arsphenamin. Do not shake it vigorously. When all is dissolved, add four drops of 15% sodium hydroxide to every .1 gm. of arsphenamin dissolved. It is necessary occasionally to add a slight excess of sodium hydroxide. The solution will at first be milky and then become clear. This solution should be alkaline to litmus paper. Filter the resulting solution with a sterile filter paper and sterile funnel. The solution is now ready for administration.

(43) PREPARATION OF NEO-ARSPHENAMIN

The apparatus is as described above for arsphenamin.

Concentration: .45 of a gram of neo-arsphenamin is generally given in 20 cc. of distilled water. It can safely be given in a smaller amount if so desired.

Dissolve the neo-arsphenamin in the water in a small bottle with a wide mouth (four-ounce bottle). Shake gently until the solution is clear. No sodium hydroxide is required for this solution. It is not generally necessary to filter. Sterile precautions are used as in making up arsphenamin.

Administration: This solution may be given with a glass syringe and needle directly or with apparatus described above for arsphenamin. If a "three-way" outfit has just been used for arsphenamin, wash it out well with salt solution before and after using the neo-arsphenamin solution, as a clouding will take place when the two solutions are mixed.

(44) INTRAVENOUS ADMINISTRATION OF ARSPHENAMIN AND NEO-ARSPHENAMIN

Apparatus:

1. Table for patient in reclining position.
2. Small table of same height about 2½ ft. square, preferably with rollers so that it is easily shifted. On the small table is a three-way stopcock fitted with three rubber tubes 40 to 50 cm. long. On the end of

INTRAVENOUS ADMINISTRATION OF ARSPHENAMIN AND NEO-ARSPHENAMIN—*continued*

one tube (*a*) is a 30 cc. glass syringe. On the other tube (*b*), an adapter for intravenous needle, and the third tube (*c*) is placed in the salt solution or the arsphenamin solution described below.

3. Several intravenous needles in 70% alcohol.
4. Sterile sponges.
5. Tourniquet to be held with a hemostat.
6. Sterile 500 cc. graduated flask (*A*) for arsphenamin solution and also another sterile 500 cc. flask (*B*) for distilled water.

The entire apparatus used in the injection is sterile.

Proceed as follows: Put end of tube (*c*) into flask (*B*) and by proper manipulation of stopcock fill the system by the syringe on tube (*a*) with salt solution. The sterile needle is then fitted to the adapter and a little salt solution washed through, about 5 cc. being left in the syringe. Cleanse patient's arm with alcohol sponge and perform venepuncture in the usual manner. Inject into the vein 5 cc. of salt solution, to make sure that the needle is in place. Now transfer tube (*c*) to flask (*A*) and by manipulation of stopcock draw into the syringe the arsphenamin solution. All of the arsphenamin may not be given in one syringe full, but the amount can best be measured by reading the level of the arsphenamin on graduated flask. (For example, to give .3 gm. of arsphenamin pump from arsphenamin flask into the vein until the level in the flask is reduced from 500 cc. to 440 cc. or until 60 cc. have been given.)

Now switch back to flask (*B*) and inject salt solution. Withdraw the needle and press on puncture wound with sterile sponge. Collodion is then applied as after ordinary venepuncture.

Acquaint yourself with the usual types of reactions and have adrenalin and sodium bicarbonate on hand.

Before giving another injection to a patient examine the skin to detect a possible toxic rash.

(45) GRAY OIL ADMINISTRATION

Formula for Gray Oil:

Chloretone	10.
Mercury	100.
Anhydrous Lanolin	150.
Olive Oil q. s. ad.	500.

Administration: A needle not less than 1½ in. long is to be used. A 2 cc. glass syringe is loaded with 5 m. of Gray Oil and sterile needle is attached. The buttock is cleansed with alcohol sponge or bichloride solution and the needle inserted to the hilt on a level with the upper end of the intergluteal crease about opposite the most prominent part of the sacrum and two thirds of the way from the midline to the iliac crest. If the skin of the buttock is made tense with the left hand and the jab quick, the insertion is usually painless. Always pull back on the plunger a little after inserting the needle, before injection, to make sure the needle is not in a vein. Inject the 5 m. of Gray Oil, followed by a little air, to insure the full dosage. After this it is generally well to inject a little air. The area injected should be rubbed vigorously with deep pressure for several minutes. Instruct the patient to do likewise twice a day for a week. The injections are made weekly.

Before injecting another dose do three things. (1) Examine and palpate the area carefully to determine a residual. (2) Examine the mouth. (3) Elicit if possible other symptoms of toxicity, as gastrointestinal upsets and urinary disorders. Use alternate buttocks each week.

(46) INTRASPINOUS TREATMENT FOR CENTRAL NERVOUS SYSTEM SYPHILIS

Twenty minutes after the intravenous injection of arsphenamin 100 cc. of blood are withdrawn with a large Schreiber needle or a large syringe, under aseptic precautions, into a wide-mouthed four-ounce bottle. A sterile stopper is put in and covered with a sterile sponge. The bottle is now tipped up at an angle of forty-five degrees and the blood allowed to

INTRASPINOUS TREATMENT FOR CENTRAL NERVOUS SYSTEM SYPHILIS—*continued*

clot. This tipping helps in obtaining the maximum amount of serum. The bottle is placed in a refrigerator for 12 to 18 hours.

The serum after standing is fairly clear and there will generally be a yield of about 25 cc. of serum. This serum is pipetted into special sterile, rubber-capped, large centrifuge tubes and spun at high speed for 10 or 15 minutes. The clear serum from the spinning is pipetted into a similar sterile rubber-capped tube and incubated at fifty-six degrees for one half hour. It is now ready for use.

Lumbar puncture is performed as described in another section. Fifteen to thirty cc. of fluid are withdrawn, the pressure generally being reduced below 30 mm. The barrel of a 20 cc. syringe with a short rubber tube connection and needle adapter is attached to the lumbar puncture needle. The syringe is lowered and some spinal fluid runs back into it, thus displacing the air in the rubber tube. The prepared serum is now poured into the syringe and allowed to flow into the subarachnoid space by gravity. The plunger may be used lightly to aid the flow of the last 5 cc. of serum. The amount of serum given depends upon the case and upon the amount of spinal fluid withdrawn. Generally 15 to 25 cc. of serum are given. The patient should remain flat on his back without pillows for 2 or 3 hours after treatment. He should remain in the hospital 12 to 24 hours after treatment, preferably in bed.

(47) SACRAL INJECTIONS

Apparatus: Hypodermic syringe with 1% novocaine; 30 cc. syringe with tubing and connecting piece to needle; lumbar puncture needle, sterile Ringer's solution.

The patient assumes the position as for a lumbar puncture. The coccygeal region is surgically cleansed. When the coccyx is pushed forward a depression is felt between it and the sacrum. The left thumb-nail palpates this depression and with the right hand insert the needle. Its course is almost

SACRAL INJECTIONS—*continued*

parallel to the sacrum. Insert about two inches. Inject into the epidural space slowly as much sterile Ringer's solution as possible up to 150 cc. If much pain is experienced, discontinue. If the subcutaneous tissues swell, the needle is not in the epidural space.

(48) MENINGITIS CASES

A suspected case is admitted to the Emergency Ward, where a lumbar puncture is performed to determine whether the case should be isolated. If the fluid is at all cloudy, drain the canal and inject a slightly smaller amount of antimeningococcus serum. This is done on the assumption that most cloudy fluids are of an infectious nature, very likely of meningococcus origin, and by injecting serum at once much valuable time may be saved the patient. If the case is an early one, it is routine to inject 60 cc. of serum intravenously. The fluid withdrawn is kept warm and cultured immediately. The remainder of the fluid is then examined to determine the etiological organism.

If the case appears quite ill, it is planned to perform cistern and lumbar puncture to irrigate the subarachnoid space with Ringer's solution and to inject serum into the cistern. This, however, is done only on those cases seen by a nerve consultant.

In examining the fluid for the organism, spin a small portion of the fluid down and stain with Smith's stain, Giemsa's stain, and Gram's stain. If no organisms are found and tuberculous meningitis is considered, search for the tubercle bacilli as outlined above.

(49) MARRIOTT TEST

The patient places his lips about the mouthpiece of the empty bag and exhales into it without previously taking a deep breath. After filling the bag with air, he rebreathes the same air until the bag is empty and then exhales the same air into the bag again. The nose is held closed throughout the breathing. This is repeated five times. When the

MARRIOTT TEST—*continued*

bag is filled for the fifth time, clamp the outlet. It is important that the circuit should be closed during the collection of the alveolar air. The small test-tube of the Marriott set is half filled with the indicator solution and the air bubbled through the solution until no further change in color occurs. The resulting color is matched against the standard colors in the Marriott apparatus.

(50) VON PIRQUET TEST

The superficial layer of the skin is broken by twisting a small "scarifier" in three places located in a line usually on the anterior side of the forearm. A match stick is moistened with tuberculin and rubbed with gentle pressure into the two end marks, the center being left as a control. The test should be read after 24 to 48 hours.

(51) SUGAR TOLERANCE TEST

Prepare as for a blood sugar determination. About 8 A.M. take a routine blood sugar, then give the patient 100 gm. of glucose by mouth. It is usually dispensed in concentrated solution, which may be diluted up to 500 cc. A half hour, one and a half hours, and three hours after the ingestion of the glucose, take samples of blood. Label the bottles in numerical order and deliver them to the laboratory at once. Whatever urine is voided during the test is examined for sugar, but parallel specimens are not necessary.

Remember that not infrequently a considerable portion of the glucose remains in the stomach. If this possibility is not controlled by examination of gastric contents, interpretation of the results should be guarded.

(52) VITAL CAPACITY

The patient is instructed to take several deep inspirations and expirations, then to fill his lungs to their greatest capacity and to blow out as much as possible into the testing machine, being careful not to allow any air to escape through the nostrils or around the tube. The indicator on the machine denotes the number of cc. of air expired and is the vital capacity.

(53) CONSULTATIONS

As a general procedure it is desirable that consultations should be authorized by the Visiting Physician. In any case the consultation should be explanatory and not perfunctory (e.g. "Diagnosis and Treatment"). Hand the consultations in at the Information Desk before 10 A.M. It is desirable, if possible, that one of the House Officers should see the case for whom the consultation is requested for the service, before the visiting man sees it. Attempt to have the consultant enter his opinion directly into the records rather than on the consultation blank. Consultations should be signed with the Visiting Physician's name and initialed by the House Officer who writes it. Dental, Eye, and Ear consultations and those with members of the Staff who are not on service at that time, should be initialed by the Admitting Superintendent.

(54) TABLE OF NORMALS

Non-Protein Nitrogen	25–35 mg. per 100 cc.
Blood Urea Nitrogen	12–17 mg. per 100 cc.
Blood Uric Acid	1–3 mg. per 100 cc.
Blood Creatinin Nitrogen	.4–.5 mg. per 100 cc.
Blood Sugar	.07–.11%
Blood Plasma Chlorides	5.4–5.9 gm. per liter
Blood Plasma CO_2	60–75 volumes %
Alveolar Air CO_2 (Marriott)	40–45 mm. Hg.
Phenolsulphonephthalein elimination in 2 hours and 10 minutes	35–60%
Reticulated Erythrocytes	
Adults	.5%
Children	2%
Blood Platelets	200,000 to 400,000 per cu. mm.
Coagulation Time	6–12 minutes
Bleeding Time	2½–6 minutes
Spinal Fluid Pressure	Up to 200 mm.
" " Cell Count	0–7 per cu. mm.
" " Total Protein	20–40 mg. per 100 cc.
" " Sugar	Approximately half of the blood sugar value, average .058%
Fragility of Erythrocytes	Starts at .420%, complete at .30%
Blood, Oxygen Carrying Capacity	20 volumes %.

(55) WIDAL TEST

Three bouillon tubes are inoculated with bacillus typhosis, Para A and Para B respectively, and are incubated from 6 to 10 hours. The culture should be cloudy with organisms before using. One cc. of the patient's blood is obtained from a vein or by milking the ear. This is allowed to clot and the serum separated by standing or by short centrifugalization. A loopful of serum is placed on a slide and surrounded by nine loopfuls of culture. On the other end of the slide four loopfuls of culture are placed. The first is thoroughly mixed and a loopful of the mixture transferred to the other. This gives dilutions of 1:10 and 1:50 respectively. Cover with cover-slips. On another slide place five loops of the culture, cover and use as a control. Dilutions of 1:10 and 1:50 are likewise set up for each organism and their controls.

(56) INTRAVENOUS ADMINISTRATION OF ANTIPNEUMOCOCCIC SERUM

Before giving serum follow the directions given under Sensitization and Desensitization. Assuming these completed proceed as follows:

Boil up "three-way" outfit with needle, syringe, etc., as for any intravenous therapy. Now dilute 100 cc. serum with 100 cc. warm normal saline solution. (The serum may be given undiluted, but as a rule it is best diluted, especially the first time.) Fill the "three-way" system with warm salt and do venepuncture. Run in a little salt and then switch to serum. The first 10 cc. serum should be injected slowly, at least 10 minutes being allowed. If no reaction occurs the remainder may be given in 15 minutes. Watch for rapidity of pulse, respiratory difficulty, pallor, and cyanosis as danger signals. From 80 to 100 cc. serum is the usual dose.

Early administration of serum is the important thing to be considered. Serum is generally given when the temperature is over 102°F. The total dosage will depend on the case and nature of the response. The Senior will direct this procedure.

(57) DIET TABLES

Increase of "House Diet" should be ordered only for "private patients" or when requested by the Visiting Physician or Surgeon for special cases.

Steaks, chops, broilers, etc., may be ordered with soft solid diets, but not with house diets, except by order of the Visiting Physician or Surgeon in special cases.

Instead of the cereal listed at present for supper, some form of soup, purée, or milk soup will be served.

SPECIAL DIETS

DIABETIC:		Ordered by the Diabetic Service, served from and by the Diet Kitchen.
FIVE MEAL DIET:	BREAKFAST	Toast and butter. Wheat cereal strained, with milk or cream and sugar.
	10.30 A.M.	Crackers and milk.
	DINNER	Egg. Baked or mashed potato. Rice or macaroni, alternate. Custard, tapioca, ice cream. Bread.
	3 to 4 P.M.	Crackers and milk.
	SUPPER	Purée. Milk toast or buttered toast. Apple sauce.
ANEMIA DIET for all three meals:		Meat. All fresh vegetables possible. 40% cream. Cheese. Fruits of all sorts. Salads. Cereals, eggs.

DIET TABLES — *continued*

FAT FREE DIET:	Scraped lean beef. Breast of chicken. Vegetables, any green. Baked potato. Salads without oil dressing. Skimmed milk. Apple tapioca or baked apple. Rice or cereal for breakfast. Milk cheese. One egg for breakfast.
SALT FREE LOW PROTEIN DIET:	No meat, no salt, no canned foods. One egg per day. Three slices of bread per day. Vegetables of all kinds. Milk, three glasses per day (governed by limitation of fluids). Fruits. 40% cream — 6 oz. Tapioca puddings. Rice, macaroni.
DIARRHEA:	No soups, fish, fruit, vegetables. Stale bread, zweibach, toast. Crackers. Boiled milk. Custard. Minced chicken, scraped beef.
ENTERIC DIET:	Dr. Shattuck's forced calories or bland foods. Minced chicken. Eggs. Ice cream. Whey. Milk toast without crusts. Macaroni. Baked apple, soft part. Orange juice. Cocoa. 40% cream. Lactose or sugar ad lib. Strained cereal.
HIGH FAT DIET (seldom used):	Bacon or fat ham. Cream soups. Salads with mayonnaise. 40% cream. Graham bread.

DIET TABLES—*continued*

CONSTIPATION (seldom used):

Cereal with cream and sugar (corn meal, rye, or oats).
Soft boiled eggs.
Coarse breads.
Coarse vegetables.
Fruits of all kinds.
Bran muffins.
Plenty of fats and coarse foods.
Force liquids.
Buttermilk.

CONSTIPATION:

Usually house diet with addition of bran muffins and coarse vegetables.

SIPPY DIET FOR PEPTIC ULCER:

For 3 days: Milk and 40% cream, equal parts, 1½ oz. every hour from 7 A.M. to 7 P.M.

On the half hour, 7.30 A.M. to 7.30 P.M., alternate MgO_2—$NaHCO_3$—$NaHCO_3$ — aa gr. 10 and Bi — aa gr. 10–30.

If diarrhea develops, use less Mg and more Bi.

After 2 to 3 days add soft boiled eggs and fine cereals so that at the end of 10 days the patient is getting milk and cream every hour, three soft boiled eggs, 9 oz. cereal, about 3 oz. each feeding. Keep adding cream soups, vegetable purées, jellies, custards, soft toast, baked potato, minced chicken, etc., until the patient is on soft solid diet.

Continue the use of Bi for 8 weeks and alkaline powders for several months between feedings.

KARELL DIET (cardio-renal with œdema):

6 oz. milk at 8 A.M., 12 M., 4 P.M., 8 P.M. No other fluids.

PURIN FREE DIET (rarely used):

No meats, meat soups, peas, beans.
Cream soups.
Vegetables.
Ice cream.
Rice, jam, marmalade.

DIET TABLES—*continued*

SOFT SOLID DIET:

Cereals.
Soups.
Chicken with gravy.
Potatoes, baked, mashed, or boiled
Bread and butter.
Jellies.
Custards.
Soft puddings.
Stewed fruit.
Tea or cocoa.

HOUSE DIET

Breakfast	*Dinner*	*Supper*
Cereal.	Soup.	Soup.
Meat or fish.	Meat or fish.	Potato or
Toast.	Potatoes.	Cereal.
Coffee or tea.	Vegetable.	Toast.
	Dessert.	Cooked fruit.
	Tea.	Cocoa or tea.

INCREASE OF HOUSE DIET

For single-room patients the house diet is increased as follows:
Fruit is added to the breakfast.
A special salad and dessert to the noon meal.
Meat or an egg dish and a salad to the evening meal.
Steaks and chops are given if specially ordered.

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